PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Fabry disease due to D313Y and novel GLA mutations
AUTHORS	Koulousios, Konstantinos; Stylianou, Konstantinos; Pateinakis, Panagiotis; Zamanakou, Maria; Loules, Gedeon; Manou, Eleni; Kyriklidou, Parthena; Katsinas, Christos; Ouzouni, Alexandra; Kyriazis, John; Speletas, Matthaios; Germenis, Anastasios E.

VERSION 1 - REVIEW

REVIEWER	Eloisa Arbustini CENTRE FOR INHERITED CARDIOVASCULAR DISEASES IRCCS FOUNDATION POLICLINICO SAN MATTEO, PIAZZALE GOLGI 27100 PAVIA ITALY
REVIEW RETURNED	30-Apr-2017

GENERAL COMMENTS	In this study authors describe novel GLA pathogenic mutations and report evidence of pathogenicity of the D313Y mutation. The D313Y mutation that seems to be associated with a later-onset milder than the typical phenotype with normal lysoGb3 concentration. The study is interesting and contributory in the setting of AFD. Comments and queries Design of the study Authors should describe the criteria used to clinically select these patients. Is the series from a population screening study of patients who demonstrated phenotypic traits suggestive for AFD? Please add the overall design of the study in methods. The quality of figure 1 should be improved, in particular, panels D. The quality of figure 3 should be improved: panel B is too dark. Figure 2: please add the triplet (wild type and mutated) on the top of each panel to facilitate readers. The mutation p.(Cys94Ser) is not novel. Please Check. Blaydon D, Hill J, Winchester B. Fabry disease: 20 novel GLA mutations in 35 families. Hum Mutat. 2001 Nov;18(5):459. The mutation p.(Tyr151X) is not novel. Please check. Shabbeer J, Robinson M, Desnick RJ. Detection of alpha-galactosidase a mutations causing Fabry disease by denaturing high-performance liquid chromatography. Hum Mutat. 2005 Mar;25(3):299-305.

REVIEWER	Joao Bosco Pesquero Universidade Federal de São Paulo Brazil
REVIEW RETURNED	28-May-2017

GENERAL COMMENTS	General evaluation The paper by Konstantinos Kouousion and collaborators is sound and describes an intriguing aspect concerning the pathogenicity of the D313Y variant in the GLA gene causing Fabry disease. Despite some individuals carrying this variant are healthy, others have clinical signs of Fabry disease. In this work the authors report five novel GLA mutations that can be result in Fabry disease, six patients carrying four previously described mutations and in other seventeen individuals with suspected of Fabry disease the mutation D313Y was found. In order to achieve the data presented, the authors analyzed samples from individuals selected by genotyping, enzymatic activity evaluation of alpha-Gal, levels of Gb3 and lyso-Gb3 and sequencing of GLA gene. For some individuals optical and electron microscopy were performed for the study of kidney biopsies. In addition, these parameters were evaluated together with clinical assessment. Major criticisms - The paper has outdated data. In the introduction the authors describe that more than 600 GLA mutations are identified and cite a 2011 paper as a reference. However, today more than 900 mutations have been described in GLA gene (http://www.hgmd.cf.ac.uk/). This outdated reflects in the results presented in the manuscript. One of the five new mutations described by the authors has already been reported in the literature. In 2005 Shabbeer et al reports the mutation p.Y151X (c.453C>G) in a female proband with Fabry disease (Detection of alpha-galactosidase A mutation causing Fabry disease by denaturing high performance liquid chromatography, Hum Mutat. 25(3):299-305). - The authors described 17 patients presenting the D313Y variant. Only five patients are classified with definitive diagnosis of Fabry disease, according to the criteria described by Biegstraaten et al. In the other patients, two do not have signs of FD, eight have some symptoms but cannot be classified as FD and the remaining two D313Y mutation carriers are apparently healthy. The authors performed enzymatic activity and quantification of lyso-Gb3 in all patients but the results are not described. It is unclear whether all individuals bearing the D313Y mutation exhibit low enzymatic activity and Gb3 accumulation. - Although five patients show signs of FD, 12 other individuals carry the same mutation and have no symptoms. These results lead to doubts about the impact of the mutation on the enzyme activity or in the etiology of the disease. As mentioned by the authors, contradictory results about the pathogenicity of the D313Y mutation have been reported in the literature. Therefore, in order to conclude that this mutation is causing Fabry disease more evidences are necessary. - Therefore, despite sound and interesting, this work does not present enough evidence to classify the variant D313Y as pathogenic. Minor criticisms - The equipments and methods used to sequence the patients are not cited. In addition, no reference to the biological sample evaluated was made.

	- The primers used to PCR the GLA gene and to sequence the exons were not described - Considering an Ion PGM sequencer has been used by the authors, why only the novel variants were verified by the Sanger sequencing method? - No references (or very few) were cited in this section. - Why the control population for the D313Y variant was Greek? All the patients presenting this variant belong to Greek population? - How was Gb3 quantified in the urine? No details about the enzymatic activity of alpha-Gal performed by mass spectrometry was described. - Abstract: please correct “sings” to “signs” (line 43). - Line 235 – Please correct “chronic chough” to “chronic cough”. - Abbreviations: VUS (variant of unknown significance) was not used in the text (Line 331).

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: The quality of figure 1 should be improved, in particular, panels D.
ANSWER. We made our best.

Reviewer 1: The quality of figure 3 should be improved: panel B is too dark.
ANSWER. We made our best.

Reviewer 1: Figure 2: please add the triplet (wild type and mutated) on the top of each panel to facilitate readers.
ANSWER. The figure has been completely rearranged.

Reviewer 1: The mutation p.(Cys94Ser) is not novel. Please Check.
ANSWER. Actually, there are 5 previous reports of the C94S mutation of in fabry-database.org. However, these reports refer to the c.281G>C (nucleotide change TGT → TCT, cDNA base 281) and not to the c.280T>A (nucleotide change TGT → AGT, cDNA base 280) detected in our study. Despite that these two nucleotide changes result in the same amino acid change (C94S) cannot be considered as the same mutation because their effect on the transcription could be different. Therefore, our c.280T>A (p.Cys94Ser, C94S) is a indeed a novel mutation. Moreover, according to ACMG guidelines, the previously reported pathogenicity of c.281G>C mutation is a strong criterion favoring the pathogenicity of another change (like the c.280T>A) in the same amino acid: [Strong criteria for classifying pathogenic variants: Same amino acid change as a previously established pathogenic variant regardless of nucleotide change].

Reviewer 1: The mutation p.(Tyr151X) is not novel. Please check.
ANSWER. Correct. Our mistake was resulted from the wrong report of the mutation detected by Shabbeer et al in fabry-database.org. We have made the appropriate corrections throughout the text especially in regard with the numbers of patients and the description of the family.

Reviewer 2: The paper has outdated data. In the introduction the authors describe that more than 600 GLA mutations are identified and cite a 2011 paper as a reference. However, today more than 900 mutations have been described in GLA gene (http://www.hgmd.cf.ac.uk/).
ANSWER. Corrected accordingly with the relevant reference.

Reviewer 2: One of the five new mutations described by the authors has already been reported in the literature. In 2005 Shabbeer et al reports the mutation p.Y151X (c.453C>G) in a female proband with Fabry disease (Detection of alpha-galactosidase A mutation causing Fabry disease by denaturing high performance liquid chromatography, Hum Mutat. 25(3):299-305).

ANSWER. Correct. Our mistake was resulted from the wrong report of the mutation detected by Shabbeer et al in fabry-database.org. We have made the appropriate corrections throughout the text especially in regard with the numbers of patients and the description of the family.

Reviewer 2: The authors described 17 patients presenting the D313Y variant. Only five patients are classified with definitive diagnosis of Fabry disease, according to the criteria described by Biegstraaten et al. In the other patients, two do not have signs of FD, eight have some symptoms but cannot be classified as FD and the remaining two D313Y mutation carriers are apparently healthy. The authors performed enzymatic activity and quantification of lyso-Gb3 in all patients but the results are not described. It is unclear whether all individuals bearing the D313Y mutation exhibit low enzymatic activity and Gb3 accumulation.
ANSWER. A clear description was added.

Reviewer 2: Although five patients show signs of FD, 12 other individuals carry the same mutation and have no symptoms. These results lead to doubts about the impact of the mutation on the enzyme activity or in the etiology of the disease. As mentioned by the authors, contradictory results about the pathogenicity of the D313Y mutation have been reported in the literature. Therefore, in order to conclude that this mutation is causing Fabry disease more evidences are necessary.
ANSWER. We completely agree and this was the main aim of our publication.

Reviewer 2: Therefore, despite sound and interesting, this work does not present enough evidence to classify the variant D313Y as pathogenic.
ANSWER. Absolutely right. Because of that nowhere throughout our manuscript we claim that D313Y variant is pathogenic. Instead, we tried to elevate the awareness of the scientific community about its possible association with pathogenicity.

Reviewer 2: The equipments and methods used to sequence the patients are not cited. In addition, no reference to the biological sample evaluated was made.
ANSWER. Described in details in the Methods section (Genotyping)

Reviewer 2: The primers used to PCR the GLA gene and to sequence the exons were not described
ANSWER. Added in Supplementary Material.

Reviewer 2: Considering an Ion PGM sequencer has been used by the authors, why only the novel variants were verified by the Sanger sequencing method?
ANSWER. Because the NGS platform we used was standardized in regard with the detection of reported variants.

Reviewer 2: No references (or very few) were cited in this section.
ANSWER. It is about the detailed description of home-made methods.

Reviewer 2: Why the control population for the D313Y variant was Greek? All the patients presenting this variant belong to Greek population?
ANSWER. Yes.

Reviewer 2: How was Gb3 quantified in the urine? No details about the enzymatic activity of alpha-Gal performed by mass spectrometry was described.
ANSWER. Appropriate references were added.

Reviewer 2: Abstract: please correct “sings” to “signs” (line 43).
ANSWER. OK.

Reviewer 2: Line 235 – Please correct “chronic chough” to “chronic cough”.
ANSWER. OK.

Reviewer 2: Abbreviations: VUS (variant of unknown significance) was not used in the text (Line 331).
ANSWER. Deleted.

VERSION 2 – REVIEW

REVIEWER	Eloisa Arbustini Centre for Inherited Cardiovascular Diseases IRCCS Foundation Policlinico San Matteo, Piazzale Golgi 27100 Pavia Italy
REVIEW RETURNED	20-Jun-2017

GENERAL COMMENTS	Authors made all requested changes. I have no further comments.

REVIEWER	Joao Bosco Pesquero Universidade Federal de São Paulo, Brazil
REVIEW RETURNED	07-Jul-2017

GENERAL COMMENTS	The authors have presented the revised version of the manuscript and addressed all the concerns this reviewer has raised. The manuscript is now acceptable for publication in BMJ Open.